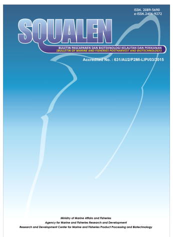

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Growth Hormone, Nitrogen and Potassium Content in the Formulated Solid Waste from Agar Processing for Fertilizer Application

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Abstract

The solid waste from agar processing industries in Indonesia is enormous. The waste contains numbers of macro and micro nutrient including growth hormone that can be a good source for plant fertilizer. Utilization of solid waste obtained from agar processing into fertilizer has been carried out by mixing with *Sargassum* powder, fish silage and *Eucheuma cottonii* pasta. Various concentrations of the solid waste (13, 19, 22 and 25%) and the *Sargassum* powder (0, 3, 6, 9, and 12%) were used in this study to obtain optimum nutrients in the formulated fertilizers i.e. growth hormone, total nitrogen and potassium. Growth hormone were analyzed using high-performance liquid chromatography (HPLC), while N content by Kjeldahl methods and K content analyzed according AOAC.The results showed that the optimum formulation was obtained from the combination of 25% solid waste and 0% *Sargassum* powder, which contained the highest growth hormones i.e. auxins (IAA) 46.75 ppm, gibberellin (GA3) 22.25 ppm, cytokinin-zeatin 18.50 ppm, and cytokinin-kinetin 61.00 ppm. While the potassium content was 0.43±0.001 ppm and the total nitrogen was 0.53± 0.06%. This result meets the requirements of the Minister of Agriculture Regulation No. 261/KPTS/SR.310/M/4/2019 regarding the requirements for total N in organic fertilizer. However, the total K^+ does not meet the requirement. Therefore, enrichment with other organic raw material is still needed.

Keywords: solid waste, agar processing, growth hormone, fertilizer

1. Introduction

The total solid waste produced annually by agar processing industries in Indonesia is averagely 29,088 MT/Y. This waste has been reported to contain high micro and macro nutrients (Munifah & Irianto, 2018) as well as growth hormones i.e. 191 ppm of auxins, 509.5 ppm of gibberellin, 244.5 ppm of cytokinin-kinetin and 70.5 ppm of cytokinin-zeatin (Basmal, Munifah, Rimmer, & Paul. 2019). Growth hormones play important roles in development of cultivated plants (Gillaspy, Ben-David, & Gruissem, 1993; Srivastava & Handa, 2005). Cytokinins (CKs) are the key regulators of various aspects in growth and development of plants, including in cell division, leaf senescence, apical dominance, lateral root formation, stress tolerance, and nutritional signalling. Meanwhile auxins are the promotor of cells division, stem and root growth involved in phototropism, geotropism, hydrotropism and other various developmental changes (Werner et al., 2003; Sakakibara, 2006; Argueso, Ferreira & Kieber, 2009).

Various minerals are found in the solid waste from agar processing. It is not only come from the *Gracilaria* as raw material but also from some additives used during the agar processing, such as perlite. This additives contain some minerals that are beneficial for plants growth such as sodium, potassium, aluminum and silicate (Anon., 2018). Silber, YosefI, Levkovitch & S.Soryano (2010) reported the effects of chemical composition in perlite which were found significant to boost plants growth, especially in enhancing the water-soluble P, Ca and Mg and in releasing the nutritional elements such as Ca, Mg, K and P. Furthermore, the use of perlite mixed with other organic materials was more effective compared to the addition of just perlite alone. The presence of other organic materials in perlite increase the pH value during the storage process due to the decomposition of organic matter. However, Sager, Park, & Chon (2007)

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reported the addition of perlite in plant media has no significant effect on crop yields and silicate tends to decrease elements such as Fe and Mn hydroxide from plant media by siderophilic bacteria.

To produce fertilizers by using solid waste from agar, some other materials are still needed particularly the nitrogen source. This efforts is conducted to meet the government regulation on fertilizer standardization, which requires minimum levels of nitrogen, phosphorus and potassium. Macronutrient K+ is one of the most essential compound in plant growth and development (Hafsi, Debez, & Abdelly, 2014; Leigh & Wyn Jones 1984; Marschner 1995; Schachtman & Shin 2007; Wang & Wu 2013). Cation is abundant in plant and is associated or involved in several physiological processes supporting the growth and development of crop (Pettigrew, 2008). The presence of the K+ is important in photosynthesis, osmoregulation, enzyme activation, protein synthesis, ion homeostasis, and the maintenance of anion-cation balances in plants (Bhandal & Malik, 1988; Marschner 1995; Zhao et al., 2001; Kanai et al., 2007).

Regulation on organic fertilizer has been released under the Minister of Agriculture Regulation No. 261 / KPTS / SR.310 /M/4/2019 regarding the technical requirements for organic, biological and soil fertilizer. Organic fertilizer should contains minimum 2% of total N + P_2O_5 + K_2O, while the compositions of hormones is not regulated. However, in some commercial organic fertilizers, synthetic growth hormones have been added to the fertilizer. To fullfill the requirements for total N and K in organic fertilizers, the formula needs to be supplied from other sources, such as fish silage. Sinclair (1990) reported that the lack of N nutrients in plants has resulted in decreasing biomass production which associated with a reduction in leaf area and its photosynthesis capacity. Zhao, Reddya, Kakania, & Reddyb (2005) reported that the leaf area and leaf photosynthetic rates are directly associated with biomass production.

Aims of this research was to obtain the best formulation of plant fertilizer from solid waste from agar processing. The formula is based on the result of previous research using solid waste of agar processing (Basmal, Hermana, Sardino, 2016a). In this research, improvement of plant growth regulators (PGRs) i.e. auxin, cytokinin, and gibberellin as main parameters in addition to macronutrients (N and K) were conducted. In addition, we used *Euchema cottonii* paste as a binder.

2. Materials and Methods

Materials used in this research were solid waste from agar processing, *Sargassum* powder, *E. cottonii* paste, and fish silage. The solid waste was obtained from *Gracilaria* agar processing factory PT. Agarindo Bogatama, Tanggerang-Banten, Indonesia. The waste was sun-dried to reach a moisture content of approximately 20% (w/w). Fresh *Sargassum* was obtained from Binuanguen Beach – West Java, Indonesia and it was sun-dried up to 15% moisture content, then ground into powder. Silage was made by modified method of fish silage (Basmal et al, 2016) from fermented trash fish using technical grade of phosphoric acid. The fish was put in a plastic drum and the phosphoric acid was added to obtain a pH value of 4. It was then fermented for 7 days until transformed into a ready-to-used silage. The *E.cottonii* paste was made by washing a dried *E.cottonii* with fresh water, then soaking overnight in freshwater. Next, it was boiled in water with a ratio of 1:30 (dried *E.cottonii* and water) until became paste.

All ingredients (Table 1) was mixed and stirred until were perfectly mixed. This mixture was then made into granules using a pellet machine. Meanwhile, to reduce the moisture content, the granules was sundried until the appropriate moisture content in range of 4.0 – 7.8% were achieved. The ready-made solid fertilizer was put into a plastic bag for further analysis of the quality of the granule fertilizer. The process of making solid fertilizer is done 2 times. Experiment has been designed based on the composition of the high PGRs content in solid waste from agar processing. The use of high PGRs can cause abnormal plant growth such as the high stems of a plant caused by high gibberellin content. Therefore it is necessary to reduce gibberellins by formulating them with other organic materials. The experiment design is presented in Table 1, and the experimental data was obtained by average of three replications.

The parameters observed in the formulated fertilizers were growth hormones (auxin, cytokine and gibberellin) using HPLC waters 2487 with detector UV-Vis (Linskens & Jackson, 1987), nitrogen content by Kjeldahl methods (BSN, 2006), and potassium content (AOAC, 2002).

3. Results and Discussion

3.1. Growth Hormones

The growth hormones analyzed in this study were auxin, gibberellin, and cytokinin. These three growth booster substances are very important for plant productivity.

3.1.1. Auxins

Fig. 1 shows IAA content in the formulated fertilizer ranged from 31.07 to 46.75 ppm with the highest value

Table 1. The design experiment of fertilizer

Raw materials	P1	P2	P3	P4	P5
Solid waste from agar processing	25%	22%	19%	16%	13%
Sargassum powder	0%	3%	6%	9%	12%
E. Cottonii paste*)	73%	73%	73%	73%	73%
Silage	2%	2%	2%	2%	2%
Total	100%	100%	100%	100%	100%

Note: *) = *E.cottonii* paste 73% equivalent with 5.5% dried *E.cottonii*

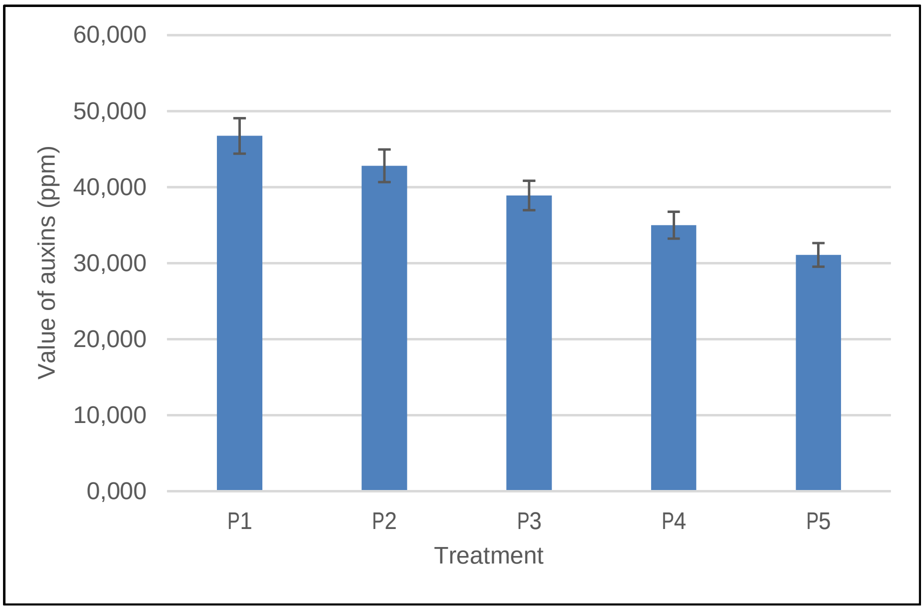

Figure 1. Auxins content in solid fertilizer made from solid waste from agar processing

Note:

a. P1: *Gracilaria* solid waste 25%, *Sargassum* powder 0%, E. *cottonii* paste 73%, and silage 2%
b. P2: *Gracilaria* solid waste 22%, *Sargassum* powder 3%, E. *cottonii* paste 73%, and silage 2%
c. P3: *Gracilaria* solid waste 19%, *Sargassum* powder 6%, E. *cottonii* paste 73%, and silage 2%
d. P4: *Gracilaria* solid waste 16%, *Sargassum* powder 9%, E. *cottonii* paste 73%, and silage 2%
e. P5: *Gracilaria* solid waste 13%, *Sargassum* powder 12%, E. *cottonii* paste 73%, and silage 2%

was found in (P1) for 46.75 ppm, and successively decreased as P2 <P3 <P4 <P5. The lowest value was 31.074 ppm found in P5 . It means by adding solid waste from agar processing up to 25% the total IAA obtained more than Arniputri et al (2003) report. Although PGRs requirements are not specifically stated in the Minister of Agriculture's regulations No. 261 / KPTS / SR.310 /M/4/2019, Arniputri et al. (2003) *in* Yunus, Rahayu, Samanhudi, Pujiasmanto, and Riswanda (2016) reported that the addition of IAA up to 1.5 ppm will increase the amount of turmeric root but tends to have a short root.

Our previous study showed that the content of auxin in the solid waste from agar processing was 191 ppm (Basmal, Munifah, Rimmer, & Paul, 2019) while in the *Sargassum* powder in range of 117.36 – 127.48 ppm (Basmal, Kusumawati, & Utomo, 2015). In the fertilizer P1, the amount of solid waste from agar processing was 25% and the *Sargassum* powder was 0%. It can be concluded that the auxin present in P1 was purely from the solid waste from agar processing. In the subsequent treatments, the amount of solid waste was smaller than that of treatment P1. However, the auxin content did not increase significantly. It means that when *Sargassum* powder was added, the auxin was loss due to the drying process that can degrades the hormone content of seaweed (Pramanik & Mohapatra, 2017).

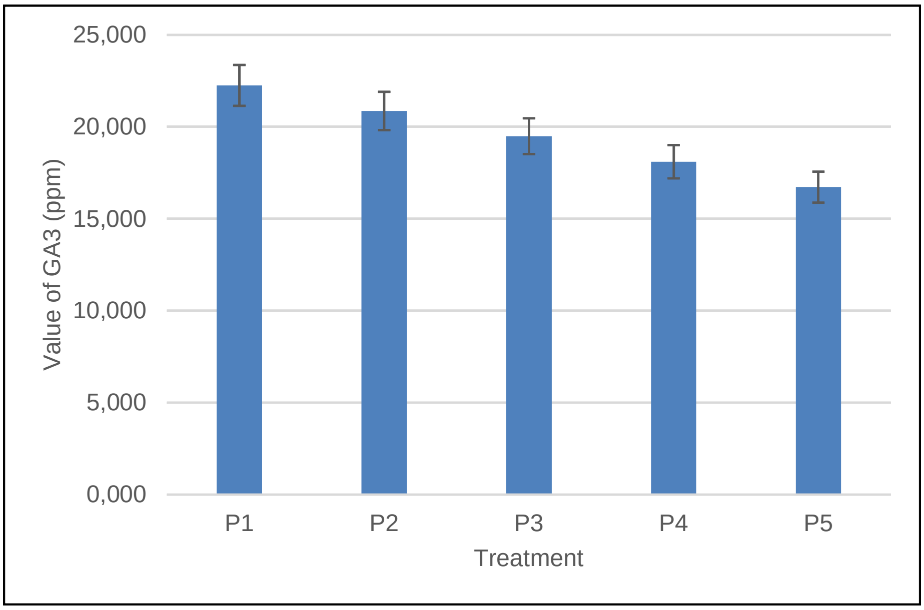

Figure 2. Gibberellin content in solid fertilizer made from solid waste from agar processing

Note:

a. P1: *Gracilaria* solid waste 25%, *Sargassum* powder 0%, E. *cottonii* paste 73%, and silage 2%
b. P2: *Gracilaria* solid waste 22%, *Sargassum* powder 3%, E. *cottonii* paste 73%, and silage 2%
c. P3: *Gracilaria* solid waste 19%, *Sargassum* powder 6%, E. *cottonii* paste 73%, and silage 2%
d. P4: *Gracilaria* solid waste 16%, *Sargassum* powder 9%, E. *cottonii* paste 73%, and silage 2%
e. P5: *Gracilaria* solid waste 13%, *Sargassum* powder 12%, E. *cottonii* paste 73%, and silage 2%

External factors that has damaging effect on phytohormone (auxin, gibberellins, cytokines) are temperature, sunlight, pH, moisture content and nutrients present in the formulation of ingredients. In this study, the key factor was the composition of the formulations. Stirk et al (2004), Nissim-Levi et al (2013) and Dubas et al (2014) reported that high temperatures can interact with auxin which will affect the formation of plant microspores polarity. Furthermore, Stirk et al (2004) reported that phytohormone such as auxin can be changed or denatured during processing, this is because phytohormone is sensitive to changes in temperature and pH. In the treatment of P1, the auxin content of solid waste from agar processing was 191 ppm and after formulation with other fertilizer materials, the auxin value became 46.75 ppm meaning that there was a decrease in auxin value by 17%. This decrease is likely due to the addition of silage. Fish silage has a low pH so that it is possible to reduce some of the auxin.

In treatments P2 to P5, the addition of *Sargassum* powder to the solid waste from agar processing not increased but affected the auxin content compared to P1 treatment. This might be due to external factors such as the temperature of the treatment, the sun's light during drying process, and pH value which affect the he auxin content in each treatment. Auxin, gibberellins, cytokinin-kinetin and cytokinin-zeatin in *Sargassum* powder that was added to the solid waste from agar processing could not increase the phytohormone content in treatments of P2, P3, P4 and P5.

3.1.2. Gibberellins content

The gibberellin content in this experiment ranged from 16.72-22.25 ppm with the highest value found in treatment P1 and the lowest in Treatment P5 (Figure 2). The Minister of Agriculture's regulations No. 261 / KPTS / SR.310 /M/4/2019 does not specified the standard of gibberellins content in fertilizer. Wulansari et al (2016) reported that gibberellic acid (GA3) has several roles in plant growth and development including elongation of stems on dwarf plants and can increase leaf size. In plant tissue culture, GA3 is commonly added, among others, to increase cell division, accelerate cell lengthening and break dormancy. While in tetraploid taro plants which was given gibberellic acids up to 1 mg /L, more roots were formed. The content of gibberellins in this study was effected by the amount of the gibberellin content for each solid fertilizer material. The amount of the solid waste from agar processing given in treatment P1 was bigger than other treatments (P1> P2> P3> P4> P5). Gibberellins (GAs) are plant hormones that are

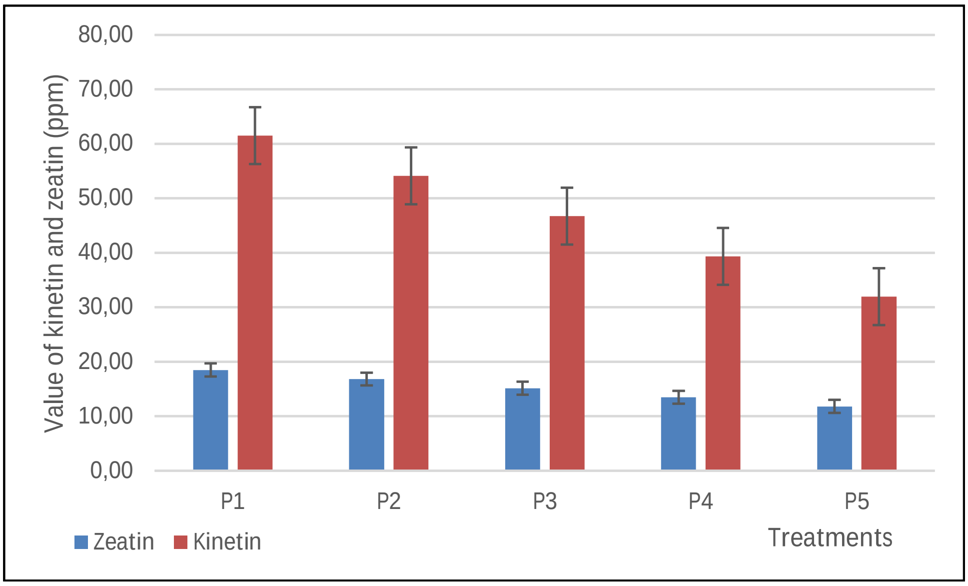

Figure 3. Cytokinin-zeatin and kinetin content in solid fertilizer made from solid waste from agar processing.

Note:

a. P1: *Gracilaria* solid waste 25%, *Sargassum* powder 0%, E. *cottonii* paste 73%, and silage 2%
b. P2: *Gracilaria* solid waste 22%, *Sargassum* powder 3%, E. *cottonii* paste 73%, and silage 2%
c. P3: *Gracilaria* solid waste 19%, *Sargassum* powder 6%, E. *cottonii* paste 73%, and silage 2%
d. P4: *Gracilaria* solid waste 16%, *Sargassum* powder 9%, E. *cottonii* paste 73%, and silage 2%
e. P5: *Gracilaria* solid waste 13%, *Sargassum* powder 12%, E. *cottonii* paste 73%, and silage 2%

essential for many developmental processes in plants, including seed germination, stem elongation, leaf expansion, trichome development, pollen maturation and the induction of flowering. Application of (GAs) to unpollinated ovaries can induce fruit set in tomato (Fos, Nuez, & García-Martínez, 2000; Serrani, Fos, Atare´s, & Garcý´a-Martý´nez, 2007; Saha, 2009; Matsuo, Kikuchi, Fukuda, Honda, & Imanishi, 2012); Daviere & Achard, 2013). Addition of *Sargassum* powder up to 12% on the treatment P5 could not increase gibberellins content compared to P1, while the results of this study found that gibberellins in solid waste from agar processing (89 ppm) was greater than *Sargassum* powder (42.89 ppm).

Each treatment was added by 2% of fish silage. The presence of weak acid derived from fish silage has caused a decrease in the amount of gibberellins in each treatment. According to Palevitch and Thomas (1976) and Mengel, Friedrich & Judel (1985) the presence of weak acids will affect the gibberellin content.

3.1.3. Cytokinin-zeatin-kinetin content

Results of the experiment showed that cytokinin zeatin in range of 11.81–18.50 ppm and cytokinin-kinetin in range of 31.98 – 61.50 ppm. The amount of cytokinin-kinetin obtained is higher than cytokinin-zeatin. Application of cytokinins (kinetin & zeatin) as much as 3 ppm is optimal, giving a positive effect on increasing the number of white turmeric shoots (Yunus et al., 2016). Furthermore, cytokinins can stimulate cell cytokenensis so that cytokinin administration can induce the formation of more shoots. Kinetin content in solid waste from agar processing was 246 ppm and liquid *Sargassum* of 84.71 ppm, while the concentration of zeatin in solid waste from agar processing was 74 ppm and *Sargassum* 18.24 ppm. Reduction of solid waste from agar processing in the formula such as P5 decreased the content of cytokinin, as well as in treatment P2, P3, P4 and P5. Matsuo et al (2012) proved that cytokine are involved in cell division during the development of tomato friut. Cytokinins are plant hormones known to be the key regulators of various aspects of plant growth and development, including cell division during embryogenesis, in the shoot apical meristem, young leaves, the cambium and cultured plant cells, leaf senescence, apical dominance, lateral root formation, stress tolerance, and nutritional signalling (Flaishman, Shargal, & Stern, 2001; Werner et al., 2003; Schmülling, 2004; Sakakibara, 2006; Argueso et al., 2009). Matsou et al. (2012) reported that cytokinin involved in early tomato fruit development. The decrease in the value of cytokinins (kinetin and zeatin) in each treatment was not only due to solid waste

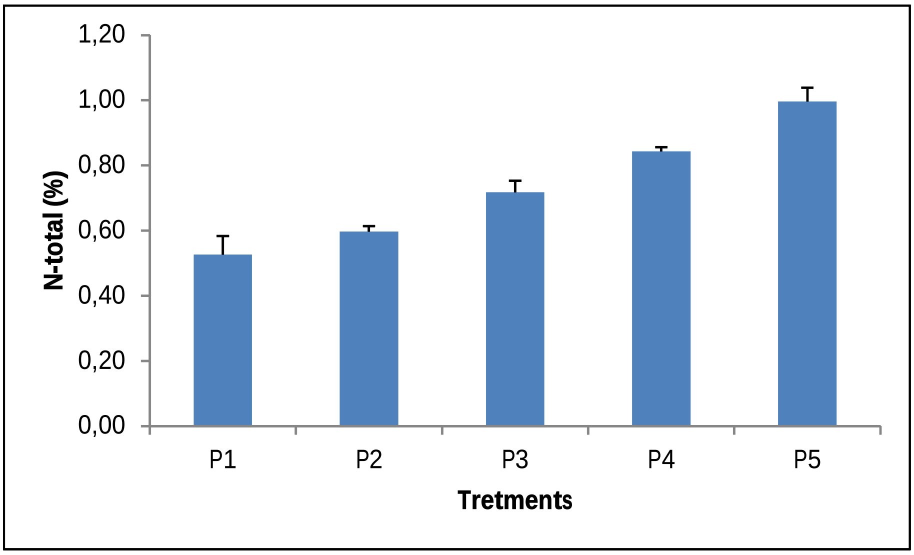

Figure 4. N total of solid fertilizer made from solid waste from agar processing

Note:

a. P1: *Gracilaria* solid waste 25%, *Sargassum* powder 0%, E. *cottonii* paste 73%, and silage 2%
b. P2: *Gracilaria* solid waste 22%, *Sargassum* powder 3%, E. *cottonii* paste 73%, and silage 2%
c. P3: *Gracilaria* solid waste 19%, *Sargassum* powder 6%, E. *cottonii* paste 73%, and silage 2%
d. P4: *Gracilaria* solid waste 16%, *Sargassum* powder 9%, E. *cottonii* paste 73%, and silage 2%
e. P5: *Gracilaria* solid waste 13%, *Sargassum* powder 12%, E. *cottonii* paste 73%, and silage 2%

from agar processing and *Sargassum* powder but might also be influenced by external factors such as temperature, humidity level, sunlight intensity during drying and internal factor combination of raw materials in each treatment given. Phytohormone decomposition can occur due to microbial activity and chemical substances loss during storage (Florence & Attwood, 1988 *in* Stirk et al., 2004). One of phytohormone, isoprenoid cytokinin, can be decereased during storage (Stirk et al, 2004). Chemical decomposition such as hydrolysis, oxidation, polymerization and isomerization can occur if the product is not stored in a dark container. The possibility of decreasing cytokinin content in all treatments could be caused by the presence of microbial activity, hydrolysis, oxidation during the formulation process. The waiting time before analysis is also one of the causes of the decrease cytokinin-zeatin-kinetin content.

3.2. N-total

The N total content in all treatments were in range of 0.53 – 1.00%. In Figure 4, it can be seen that the increase in total N levels has been influenced by the amount of *Sargassum* powder added. The higher the *Sargassum* powder, the more the N-total content (P1< P2<P3< P4<P5). N-total of Sargasum powder (1.56%) was higher than the solid waste from agar processing (0.29%) so that the increase in N-total value was effeted by the addition of *Sargassum* powder and also the presence of fish silage. The requirements for total N in solid organic fertilizer is not specifically mentioned by Minister of Agriculture Regulation No. 70 / Permentan / SR.140 / 10/2011, however, it is mention that the level of N + P_2O_5 + K_2O should at least 4% which means that the minimum N requirement in solid organic fertilizer is amounting to 0.0021%. It means that all treatments meet the requirement of the regulation.

3.3. Potassium Content

Potassium (K^+) levels in all treatments ranged from 0.43 - 4.23 mg/100g with the highest value found in treatment P5 (ratio of 13% solid waste from agar processing and 12% *Sargassum* powder) of 4.23$\pm$ 0.01 mg/100g, while the lowest was in treatment P1 (ratio of solid waste from agar processing and 0% *Sargassum* powder) of 0.43 $\pm$ 0.00 mg/100g (Figure 3). Based on these findings, the amount of *Sargassum* powder mixed into a solid waste from agar processing has increased K content in the treatment. The results of K^+ analysis on a solid waste from agar processing and *Sargassum* powder were 0.27 mg/100 g and 7.08 mg/100 g, respectively. The higher K^+ value in P2, P3, P4 and P5 treatments has been affected by the addition of *Sargassum* powder which contained more K^+, besides the addition of fish silage in the control

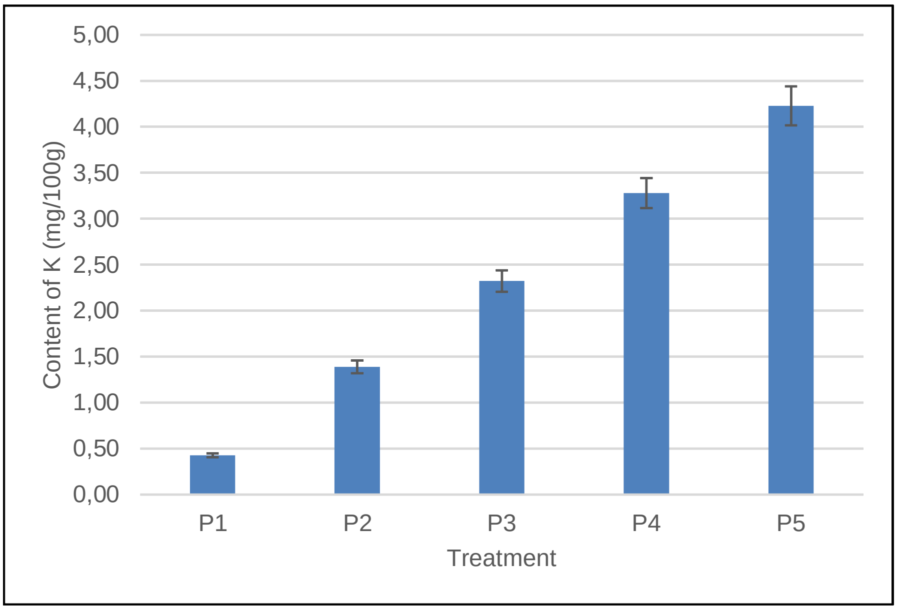

Figure 5. Potassium (K^+) content in solid fertilizer made from solid waste from agar processing

Note:

a. P1: *Gracilaria* solid waste 25%, *Sargassum* powder 0%, E. *cottonii* paste 73%, and silage 2%
b. P2: *Gracilaria* solid waste 22%, *Sargassum* powder 3%, E. *cottonii* paste 73%, and silage 2%
c. P3: *Gracilaria* solid waste 19%, *Sargassum* powder 6%, E. *cottonii* paste 73%, and silage 2%
d. P4: *Gracilaria* solid waste 16%, *Sargassum* powder 9%, E. *cottonii* paste 73%, and silage 2%
e. P5: *Gracilaria* solid waste 13%, *Sargassum* powder 12%, E. *cottonii* paste 73%, and silage 2%

treatment (P1) has also increased the potassium content. Based on the Minister of Agriculture Regulation No. 70/Permentan/SR.140/10/2011 the level of N + P_2O_5 + K_2O should at least 4% which means that the minimum requirement of K^+ in solid organic fertilizer is amounting to 230 ppm. While the results of the highest K^+ value analysis in this study was only 43 ppm, which means that the K^+ value requirements for all treatments were not achieved.

4. Conclusion

Solid waste from agar processing has the potential to be used as a fertilizer. The best results was the P1, i.e combination of 25% solid waste from agar processing (w/w) with 0% *Sargassum* powder based on the PGRs namely auxins (46.75 ppm); gibberellin (22.25 ppm), cytokinin-zeatin (18.50 ppm) and cytokinin-kinetin (61.50 ppm). The best formula contained N of 0.53%$\pm$0.06 and K^+ of 0.43$\pm$0.00 (mg/100g). This result meets the requirements determined by Minister of Agriculture Regulation No. 261 / KPTS / SR.310 /M/4/2019 concerning the requirements for total N, but not K^+. Therefore, enrichment with other organic raw material is still needed.

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